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IS 14629 (1999): Method for Determination of Dicofol Residues in Agricultural and Food Commodities [FAD 1: Pesticides and Pesticides Residue Analysis]

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कृषि एवं खाद्य पदार्थों में डाइकोफाल अवशेष ज्ञात करने की पद्धतियां

Indian Standard

METHOD FOR DETERMINATION OF DICOFOL RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES

ICS 65.100 ; 67.040

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

February 1999 **Price Group 2**

Pesticides Residue Analysis Sectional Committee, FAD 34

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Pesticides Residue Analysis Sectional Committee had been approved by the Food and Agriculture Division Council.

Dicofol formulations are used in agriculture for the control of mites. It is a non-systemic acaricide with little insecticidal activity. Although residues in soil decrease rapidly, traces may remain for more than one year.

In the preparation of this standard due consideration has been given to the limits of dicofol residues laid down under the provisions of *Prevention of Food Adulteration Act*, 1954 and Rules framed thereunder.

This standard will enable the food, health authorities and others engaged in the field to follow uniform test procedures for the estimation of dicofol and its major metabolite 4,4 - dichlorobenzophenone residues in various agricultural food commodities and soil.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHOD FOR DETERMINATION OF DICOFOL RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES

1 SCOPE

This standard prescribes the gas chromatographic method for the determination of dicofol [2,2,2 - trichloro - 1,1 - bis(4- chlorophenyl) ethanol] and its major metabolite 4,4′- dichlorobenzophenone residues in agricultural, food commodities and soil.

NOTE — The method has a detection limit 0.01 µg/g (0.01 ppm).

2 REFERENCE

The following standard contains provisions which through reference in this text, constitutes provision of this standard. At the time of publication, the editions indicated was valid. All standards are subjected to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below:

IS No.	*Title*
11380 (Part 1) : 1985	Method of sampling for the determination of pesticide residues: Part 1 Agricultural and food commodities

3 SAMPLING

The representative samples for the purpose of estimating dicofol residues shall be drawn in accordance with IS 11380 (Part 1).

4 APPARATUS

4.1 Waring Blender

4.2 Kuderna-Danish Evaporator

4.3 Centrifuge — with 250 ml capacity centrifuge bottles.

4.4 Filtering Funnel — fitted with filter having porosity rated as 1.

4.5 Soxhlet Apparatus

4.6 Sieve — 2 mm aperture size.

4.7 Glass Column — 45 cm long and 2.0 cm in dia.

4.8 Hot Air-Oven

4.9 Water Bath

4.10 Glass Wool Filter

4.11 Separatory Funnel

4.12 Rotary Evaporator

4.13 Erlenmeyer Flask

4.14 Glass Column (380 mm × 15 mm)

5 REAGENTS

5.1 Sodium Sulphate — (anhydrous) AR grade.

5.2 *n*-Hexane — all glass re-distilled HPLC grade.

5.3 Triton X-45 — 10 percent aqueous mixture (*v/v*).

5.4 Nitrogen Gas — free of impurities.

5.5 Acetone — all glass re-distilled.

5.6 Petroleum Ether — all glass re-distilled.

5.7 Acetonitrile — all glass re-distilled.

5.8 Distilled Water

5.9 Florisil

60-100 mesh, column chromatography grade. Heat at 130°C for 24 hours to remove moisture. Deactivate by adding 2.5 percent of water (2.5 ml water and 97.5 g dried florisil) and let equilibriate for 24 hours in tightly stoppered bottle before use.

5.10 Potassium Oxalate Solution

10 percent aqueous solution (*w/v*).

5.11 Dicofol Reference Standard — of known purity. Working standard solution of dicofol reference standard of known purity shall be used at the concentration of 0.1, 0.2, 0.5, 1.0 and 2.0 µg/ml in *n*-hexane.

5.12 Diethyl Ether, HPLC grade.

6 EXTRACTION

6.1 Crop Samples

Transfer a suitable quantity (100-200 g) of finely chopped crop sample into the blender jar. Add 10-50 g of anhydrous sodium sulphate followed by *n*-hexane keeping the sample-solvent ratio at 1:2 (*w/v*). Blend at high speed for 3-5 minutes. Filter the mixture through a funnel fitted with a permanent filter of the porosity rated as 1. Wash the blender jar twice with

50 ml of *n*-hexane. Combine the filtrate and washing. Concentrate the aliquot on steam bath with Kuderna-Danish evaporator. Traces can be removed at room temperature by passing a gentle stream of air.

6.2 Milk Samples

6.2.1 Either **6.2.1.1** and **6.2.1.2** may be followed for extraction of the pesticide in milk samples.

6.2.1.1 Place 100 ml of milk in a 250 ml centrifuge bottle. Add 30 ml potassium oxalate solution and warm to about 45°C on a water bath. Add 10 ml aqueous mixture of Triton X-45, mix thoroughly and maintain at 45°C for 20 minutes. Cool to room temperature. Centrifuge to obtain a clear fat layer and add 100 ml of *n*-hexane. Stopper and shake the bottle gently to gather the fat layer. Centrifuge until the *n*-hexane layer is clear. Remove the *n*-hexane layer without disturbing the milky phase. To wash over the *n*-hexane left behind, slowly add another 100 ml of *n*- hexane (do not disturb the milky phase), separate and add to the first extract. Give one more washing and combine all the extracts. Filter the extract in Kuderna-Danish evaporator and concentrate under the a nitrogen atmosphere to dryness (or fatty residues).

6.2.1.2 Add acetone to 100 ml of milk which will result in coarse precipitation of milk solids. Remove the solids by filtering through glass wool. Take the filterate in a separatory funnel and extract the pesticide twice with 100 ml of petroleum ether each time. Combine the extracts and concentrate it on water bath.

6.3 Animal Tissue

Place a 50 g sample of animal tissue in a blender. Add enough anhydrous sodium sulphate to absorb water present in tissues. Add 200 ml of *n*-hexane and blend for 5 minutes. Pour the extract in contrifuge bottle, rinsing the blender jar with 50 ml of *n*- hexane and add to centrifuge bottle. Centrifuge for 5 minutes at about 1 500 rpm. Separate the *n*-hexane layer and concentrate (use 50 ml of *n*-hexane to rinse centrifuge tubes).

6.4 Tea Sample

Take 50 g of tea and extract by blending with 150 ml *n*-hexane in a waring blender for 10 minutes. The extract is filtered through Buchner funnel with Whatman No. 41 filter paper. The filtrate thus obtained is then concentrated to 10 ml with the help of a rotary vacuum evaporator. The concentrate is freed from moisture with anhydrous sodium sulphate.

6.5 Urine Sample

Take 20 ml of urine in a separatory funnel and add 100 ml of acetonitrile and 50 ml of distilled water. Extract the mixture 3 times with 600 ml *n*-hexane each time. Combine the extractions and evaporate the solvents at 40°C on a rotary evaporator.

7 CLEAN UP

7.1 Pack a glass column to a height of about 5 cm with florisil. The column shall be saturated with petroleum ether. Add the sample extract to this column gradually. Thereafter, elute the *pp*-dichlorobenzophenone using a mixture 15 ml of ether and 135 ml of *n*-hexane. Finally, elute the *pp*-dicofol using a mixture containing 35 ml of ether and 115 ml of *n*-hexane.

The flow should be regulated at about 2 ml per minute with the help of a rubber cork.

NOTE — This method helps to separate all the important metabolites in a mixture except of course *pp′* dicofol and *pp′* - dichlorobenzophenone (*pp′* dicofol is converted into *pp′* dichlorobenzophenone immediately after injecting in gas chromatograph). The only known and easy solution to the problem is the use of a florisil column to separate those two compounds prior to column injection.

For eluting *pp′* dicofol use eluting mixture of 35 ml ether and 115 ml of *n*-hexane whereas for eluting *pp′* dichlorobenzophenone a mixture of 15 ml ether and 135 ml *n*- hexane may be used.

7.2 Tea samples are cleaned up by passing through an absorbant column packed with 20 g of activated florisil. The column is elute with a mixture of 10 percent diethyl ether in *n*-hexane (200 ml). Finally the column elute is evaporated to dryness and the final volume is made up to 10 ml with *n*-hexane.

8 ESTIMATION

8.1 Principle

8.1.1 The dicofol residue extracted from the sample is measured gas chromatographically using an electron capture detector. The content of dicofol is determined by comparing the response with a reference standard of dicofol.

8.2 Gas Chromatograph

8.2.1 A gas chromatograph equipped with an electron capture detector used for the separation and estimation of dicofol from extracts. After injecting the GLC column gets converted to corresponding ketone, which elutes from the column and gives a well defined peak. The area of ketone peak is used for quantification purpose under the following suggested conditions. The parameters may be changed according to the available facilities, provided standardization is done.

Column	—	Glass, 1.8 m long and 2 mm ID, 2 percent OV - 101 on chromosorb WHP (80 - 120 mesh)	
Detector	—	Electron capture detector	
Temperature	—	Column oven	210°C
		Injection port	260°C
		Detector	250°C
Carrier gas	—	Nitrogen 30 ml	

8.2.2 *Microlitre Syringe* — 10 µl.

8.3 Procedure

8.3.1 Suitably adjust the gas chromatograph so as to get at least 50 percent full-scale deflection with a 1 µg/injection of dicofol reference standard solution.

8.3.2 Adjust the volume of clean-up sample solution (*see* **7**) either by concentration or dilution with hexane to achieve on scale peaks. Inject 2-5 µl of the sample and identify the presence of dicofol residues by comparing the retention time of the sample peak with that of reference standard.

8.4 Calculation

$$\text{PPM of Dicofol} = \frac{A_1 \times \text{Concentration of ref standard} \times \text{Volume of sample inject}}{A_2 \times \text{Mass of the sample injected} \times F}$$

where

A_1 = peak area of the dicofol sample injected,

A_2 = peak area of the dicofol reference standard injected, and

F = percent recovery.

NOTE — Percent mean recovery is determined by taking untreated control sample to which a known amount of dicofol is added and analysed, as described above.

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Amendments Issued Since Publication

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